Amendments to the Specification:

Please add the following new paragraph beginning at page 1, line 1, immediately after the title and prior to the heading "Field of the Invention:"

Cross-Reference to Related Applications

This application is a divisional of U.S. Patent Application Serial No. 09/504,505 filed February 15, 2000, now U.S. Patent No. 6,315,998, which is a continuation of U.S. Patent Application Serial No. 08/463,893, filed June 5, 1995, now U.S. Patent No. 6,056,959, which is a divisional of U.S. Patent Application Serial No. 08/070,158, filed May 28, 1993, now U.S. Patent No. 5,677,165, which is a continuation-in-part of U.S. Patent Application Serial No. 07/910,222, filed July 9, 1992, now U.S. Patent No. 5,397,703, the disclosures of which are hereby incorporated by reference.

Please replace the paragraph beginning on page 1, immediately after the heading "Field of the Invention," with the following paragraph:

This invention relates to novel methods of treating diseases of the immune system. In particular, this invention relates to methods of preventing or treating antibody-mediated diseases such as IgE-mediated disease (allergies) and autoimmune diseases including ~~sytematic~~ systematic lupus erythematosus (SLE), primary biliary cirrhosis (PBC), and idiopathic thrombocytopenic purpura (ITP).

Please replace the paragraph beginning on page 3, line 2, with the following paragraph:

The CD40 antigen is a glycoprotein expressed on the cell surface of B cells. During B cell ~~diferentiation~~ differentiation the molecule is first exposed on pre-B cells and then disappears from the cell surface when the B cell becomes a plasma cell. Crosslinking of CD40 molecules with anti-CD40 antibodies mediates a variety of effects on B cells. The CD40 antigen is known to be related to the human nerve growth factor (NGF) receptor and tumor necrosis factor-alpha (TNF-$\alpha$) receptor, suggesting that CD40 is a receptor for a ligand with important functions in B-cell activation.

Please replace the paragraph beginning on page 3, line 26, with following paragraph:

All anti-CD40 antibodies known in the art have a stimulatory ~~efffect~~ effect on human B cells. Cross-linking of the CD40 molecule on the B-cell surface using known anti-CD40 antibodies mediates a variety of effects on B cells. Anti-CD40 monoclonal antibodies (mAbs) can induce intercellular adhesion, proliferation and, in combination with certain cytokines, maturation to antibody secreting cells. For example, known anti-CD40 mAbs have been shown to mimic the effects of T helper cells in B-cell activation. When presented on adherent cells expressing FcγRII, these antibodies induce B-cell proliferation. J. Banchereau et al., Science (1989) 251:70. Moreover, the known anti-CD40 mAbs can replace the T helper signal for secretion of IgM, IgG and IgE in the presence in IL-4. H. Gascan et al., J. Immunol. (1991) 147:8. Furthermore, known anti-CD40 mAbs can prevent programmed cell death (apoptosis) of B cells isolated from lymph nodes.

Please replace the paragraph beginning on page 5, line 12, with the following paragraph:

It is yet another object of this invention to provide a method for preventing or treating an antibody-mediated autoimmune disease in a patient, the method comprising administering to a patient in need of such treatment a therapeutically effective amount of a monoclonal antibody capable of binding to a human CD40 antigen located on the surface of a human B cell, wherein the binding of the antibody to the CD40 antigen prevents the growth or differentiation of the B cell, in a pharmaceutically acceptable excipient. Particular autoimmune diseases contemplated for treatment by this method include ~~sytematic~~ systematic lupus erythematosus (SLE), primary biliary cirrhosis (PBC), and idiopathic thrombocytopenic purpura (ITP).

Please replace the paragraph beginning on page 6, line 2, with the following paragraph:

Figure 1A shows a schematic representation of the baculoviral transfer vector pAcC8 and the sequence of the multiple cloning site (SEQ ID NO:1). As shown, the multiple cloning site was inserted between nucleotide number +37 and +176 of the polyhedrin gene. Figure 1B shows a schematic representation of the generation of Sf9 cells which express human CD40 or B7 antigen.

Please replace the paragraph beginning on page 6, line 6 with the following paragraph:

Figure 2 shows the sequences of polymerase chain reaction primers used in the preparation of coding regions for human CD40 and human B7 antigens. These primers were constructed on the basis of the published complete DNA coding sequences for antigens B7 and CD40. Specifically shown are the primers for full length B7, which are designated as MR67 (SEQ ID NO:2) and MR69 (SEQ ID NO:3); the primers for soluble B7, which are designated as MR67 (SEQ ID NO:2) and MR145 (SEQ ID NO:4); the primers for full length CD40, which are designated as MR108 (SEQ ID NO:5) and MR112 (SEQ ID NO:6); and the primers for soluble CD40, which are designated as MR108 (SEQ ID NO:5) and MR150 (SEQ ID NO:7).

Please replace the paragraph beginning on page 6, line 13, with the following paragraph:

~~Figure 4 show~~ Figures 4A-4C show the results of the fluorescent cell staining of EBV-transformed B-cell line ARC cells expressing CD40. Figure 4A shows the results of staining ARC EBV transformed cells with serum from a mouse immunized with B7 expressing Sf9 cells (solid line) or with normal mouse serum (dotted line). Figure 4B shows the results of staining ARC EBV transformed cells with serum from a mouse immunized with CD40 expressing Sf9 cells (solid line) or with normal mouse serum (dotted line). Figure 4C shows the results of staining ARC EBV transformed cells with serum from a mouse immunized with control Sf9 cells (solid line) or with normal mouse serum (dotted line).

Please replace the paragraph beginning on page 8, line 13, with the following paragraph:

The antibodies of the current invention bind to a human CD40 antigen on the surface of a human B cell and do [[not]] not simulate the growth ~~of~~ or differentiation of the B cell. These antibodies may be polyclonal antibodies, monoclonal antibodies, humanized antibodies, single-chain antibodies, and fragments thereof.

Please replace the paragraph beginning on page 14, line 13, with the following paragraph:

As stated above, the antibodies and compositions of this invention are used to treat human patients to prevent or treat antibody-mediated diseases such as allergies, SLE, PBC and ITP. The preferred route of administration is ~~parenterally~~ parenteral. In parenteral administration, the compositions of this invention will be formulated in a unit dosage injectable form such as a solution, suspension or emulsion, in association with a pharmaceutically acceptable parenteral vehicle. Such vehicles are inherently nontoxic and nontherapeutic. Examples of such vehicles are saline, Ringer's solution, dextrose solution, and Hanks' solution. Nonaqueous vehicles such as fixed oils and ethyl oleate may also be used. A preferred vehicle is 5 % dextrose in saline. The vehicle may contain minor amounts of additives such as substances that enhance isotonicity and chemical stability, including. buffers and preservatives.

Please replace the paragraph beginning on page 17, line 5, with the following paragraph:

For testing the ability of anti-CD40 mAbs to stimulate B-cell proliferation in a culture system analogous to that described by Banchereau et al. Science ~~(1989)~~ (1991) 251:70, mouse 3T6 transfectant cells expressing the HR allellic form of human FcγRII were used. B cells (2 x $10^4$ per well) were cultured in flat-bottom microwells in the presence of 1 x $10^4$ transfectant cells (irradiated with 500 Rad) in 200 µl IMDM supplemented with 10% fetal calf serum and 100 U/ml recombinant IL-4. Before addition of the B cells, the 3T6 cells were allowed to adhere to the culture plastic for at least 5 hours. Anti-CD40 mAbs were added at concentrations varying from 15 ng/ml to 2000 ng/ml and proliferation of B cells was assessed by measurement of thymidine incorporation at day 7, upon 18 hour pulsing with [$^3$H]-thymidine.

Please replace the paragraph beginning on page 18, line 28, continuing through page 19, line 6 with the following paragraph:

ARC cells ($10^6$ cells/sample) were incubated in 100µl primary antibody (10 µg/ml in PBS-BSA or Hank's balanced salt solution (HBSS) supplemented with 1% BSA and 0.05 % sodium azide) for 20 min at 4°C. After 3 washes with PBS-BSA or HBSS-BSA, the cells were incubated in 100 µl FITC-labeled $F(ab')_2$ fragments of goat anti-(mouse IgG) antibodies

(Jackson, West Grove, PA) for 20 min at 4°C. After 3 washes with PBS-BSA or HBSS-BSA and 1 wash with PBS, the cells were resuspended in 0.5 ml PBS. Analyses were performed with a FACSCAN V™ cytofluorometer (Becton Dickinson, San Jose, CA).

Please replace the paragraph beginning on page 19, line 7, with the following paragraph:

Alternatively, EL4B5 cells were harvested before and at different time points during culture in medium containing PMA (5ng/ml) and human T-cell supernatant (5%). Cells were incubated for 30 minutes with 10 µl supernatant of transfected cells containing hCD40-Hµ diluted in 100 µl Hank's Balanced Salt Solution supplemented with 0.05% sodium azide (4°C). This was followed by incubation with FITC-conjugated $F(ab')_2$ fragments of rabbit anti-(human IgM) (Central Laboratory of the Blood Transfusion Service, Amsterdam, The Netherlands). As a control, cells were incubated with the FITC-conjugate only. For analysis a FACScan-4™ cytofluorometer (Becton ~~and~~ Dickinson) was used. Non-vital cells were excluded from analysis by the use of propidium iodide.

Please replace the paragraph beginning on page 20, line 18, with the following paragraph:

For PCR amplification, 1 µl of cDNA was mixed with 1 µl (10 picomoles) of a forward primer, 1 µl (10 picomoles) of a backward primer, and 47 µl of PCR mix. The PCR mix consisted of 1.25 units Taq polymerase (Perkin-Elmer/Cetus, Norwalk, CT), dNTP mix (0.2 mM each), 10 mM ~~Tris-cHL~~ Tris-HCl pH 8.3, 50 mM KC1, 2.5 mM $MgC1_2$ and 0.1 mg/ml BSA. The 50 µl of PCR mixture was overlaid with 70 µl mineral oil and subjected to 25 cycles of amplification in a Perkin-Elmer/Cetus thermocycler (denaturation at 95°C for 30 seconds, primer annealing at 55°C for 30 seconds and extension at 72°C for 1.5 minutes). PCR products were obtained after 25 amplification cycles.

Please replace the paragraph beginning on page 22, line 2, with the following paragraph:

Sf9 insect cells infected with recombinant virus were cultured for 48 hours in 24-well plates. After removal of the tissue culture medium the plates were incubated for 45 minutes at room temperature (RT) with 0.25 ml of antibody in PBS with 1% BSA (PBS-BSA). After three

~~washed~~ washes with PBS-BSA, the plates were incubated for 35 minutes at RT with 250 µl of a 1/250 dilution of goat anti-(mouse total Ig) immunoglobulins conjugated to horseradish peroxidase (Zymed, South San Francisco, CA) in PBS-BSA. Unbound peroxidase activity was removed by washing five times with PBS-BSA. Bound peroxidase activity was revealed by the addition of an assay mixture prepared by diluting 0.5 ml of 2 mg/ml 3,3',5,5'-tetramethylbenzidine in ethanol to 10 ml with 10 mM sodium acetate, 10 mM EDTA buffer (pH 5.0) and adding 0.03% (v/v) $H_2O_2$. The reaction was stopped after 10 minutes by adding 100 µl of 1 M $H_2SO_4$.

Please replace the paragraph beginning on page 23, line 2, with the following paragraph:

Splenocytes from immunized BALB/c mice were fused with SP2/0 murine myeloma cells at a ratio of 10:1 using 50% polyethylene glycol as previously described by Boer et al., J. Immunol. Meth. (1988) 113:143. The fused cells were resuspended in complete IMDM medium supplemented with hypoxanthine (0.1 mM), aminopterin (0.01 mM), thymidine (0.016 mM) and 0.5 ng/ml hIL-6 (Genzyme, Cambridge, MA). The fused cells were then distributed between the wells of 96-well tissue culture plates, so that each well contained 1 growing ~~hybrid~~ hybridoma on average.

Please replace the paragraph beginning on page 24, line 3, with the following paragraph:

Four hybridomas producing monoclonal antibodies against human CD40 were generated in Example 1. These mAbs were shown to bind to a similar proportion of tonsillar B cells as anti-CD40 mAb G28.5 does. De Boer et al. J. Immunol. Methods (1992) 152:15. Three of these monoclonal ~~antiodies~~ antibodies (5D12, 3A8 and 3C6) which were the IgG2b subclass, were tested for their ability to deliver activation signals to human B cells in the B-cell proliferation assay described above.

Please replace the paragraph beginning on page 25, line 17, with the following paragraph:

It was found that each of the anti-CD40 mAbs 5D12, 3A8 and 3C6 could inhibit the costimulation of anti-IgM induced human B-cell proliferation by mAb S2C6 (Figure 6). In

contrast, no significant inhibition was seen with equivalent amounts of non-relevant mAb 8E4, directed to ß-glucocerebrosidase. Barneveld et al., supra. Thus, it was concluded that these anti-CD40 mAbs do not deliver stimulatory signals ~~to~~ for the proliferation of human B cells, but conversely, can inhibit stimulatory signals exerted by triggering ~~anti-CD40~~ CD40 with another mAb. Therefore, these mAbs were considered to be excellent tools to investigate whether signaling via CD40 plays a role in the stimulation of human B-cell proliferation by EL4B5 cells.

Please replace the paragraph beginning on page 26, line 6, continuing through page 27, line 4 with the following paragraph:

For comparison, the influence of a few mAb directed against other B-cell surface structures was investigated. Neither anti-CD20 mAb B1 or anti-B7 mAb B7-24 (the latter mAb was generated by a procedure similar to that used for generating the anti-CD40 mAB used in Figure 7[[,]] ) in concentrations similar to those used in the experiments with the anti-CD40 mAb, had any effect on EL4B5-induced human B-cell proliferation (data not shown). Therefore, it may be concluded that the inhibitory effect of anti-CD40 mAb on ELAB5-induced B-cell proliferation is not due to masking of the B-cell surface.

Please replace the paragraph beginning on page 29, line 2, with the following replacement paragraph:

The hybridomas used in the above examples, to illustrate the method of the present invention were deposited in and accepted by the American Type Culture Collection (ATCC), ~~12301 Parklawn Drive, Rockville, Maryland,~~ 10801 University Boulevard, Manassas, VA USA, under the terms of the Budapest Treaty.

| Hybridoma | Deposit Date | Accession No. |
|---|---|---|
| B7-24 | May 6, 1993 | HB 11341 |
| 3C6 | May 6, 1993 | HB 11340 |
| 5D12 | May 6, 1993 | HB 11339 |
| 3A8 | January 30, 1996 | HB 120124 |